Papers

Investigation of risk factors for *Salmonella* on commercial egg-laying farms in Great Britain, 2004-2005

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In 2004/05, all European Union member states were required to carry out standardised prevalence surveys to establish the baseline prevalence of *Salmonella* in commercial laying flocks. As part of the survey in Great Britain, additional data were collected from 380 of the enrolled laying hen holdings to investigate risk factors for *Salmonella* at farm level. Stratified, simple random sampling was used to select holdings from which dust and boot swab samples were collected and tested for *Salmonella* using a modification of ISO 6579:2002. Using a multivariable logistic model weighted to account for the survey design, several factors significantly associated with *Salmonella* and *Salmonella* Enteritidis status were identified. Larger holdings (≥30,000 birds) were found to be at higher risk of *Salmonella* (odds ratio [OR] 4.79, P=0.025), while vaccination (OR 0.28, P=0.013), providing foot dips with brushes (OR 0.27, P=0.042), washing and disinfecting the house at depopulation (OR 0.19, P=0.003), having a clean car park away from house (OR 0.14, P=0.001), using an independent (OR 0.19, P=0.007) or other non-company (OR 0.40, P=0.049) source of feed, being over 1 km from the nearest neighbouring farm (OR 0.45, P=0.021) and the presence of cats and dogs on the farm (OR 0.26, P=0.002) or on contiguous farms (OR 0.44, P=0.030) reduced the risk of any *Salmonella* serovars being present. Factors found to be associated specifically with an increased risk of *S* Enteritidis infection included holding size (OR 14.88, P=0.001) and frequent sightings of rats (OR 8.17, P<0.001) or mice (OR 5.78, P=0.006). Non-caged systems (OR 0.14, P=0.002), vaccination (OR 0.08, P=0.001), the use of a non-company feed source (OR 0.11, P=0.003), running the site as all-in/all-out (OR 0.06, P<0.001) and the presence of cats and dogs on the farm (OR 0.14, P=0.002) were associated with a reduced risk.

SINCE the peak of the *Salmonella* epidemic in the late 1990s, cases of human salmonellosis in England and Wales have decreased by 64 per cent, to 11,350 laboratory-confirmed cases in 2005 (Anon 2006a). The epidemic was largely dominated by *Salmonella* Enteritidis PT4, which accounted for 70 per cent of cases in 1997 (Cogan and Humphrey 2003). Since then, this phage type has shown a marked decline, to be replaced with other previously less common phage types (Anon 2004a). In 2005, only 16 per cent of human cases of salmonellosis were due to *S* Enteritidis PT4 infection, and 13 per cent were attributed to *Salmonella* Typhimurium (Anon 2006a). Most cases of *S* Enteritidis infection in human beings in the UK are still thought to be associated with the consumption of contaminated and insufficiently cooked egg products (Hogue and others 1997, Kessel and others 2001), and salmonellosis still constitutes an important public health concern in the UK (Roberts and Socket 1994, Adak and others 2002). The observed reduction in the number of UK cases is largely due to efforts by the poultry industry to reduce the prevalence of *Salmonella* at farm level, and thus minimise the risk of infection to human beings through contaminated eggs and broiler meat. Statutory monitoring and control of *S* Enteritidis and *S* Typhimurium, including implementation of the 1993 Poultry Breeding and Hatcheries Order (Anon 1993) and improved on-farm measures such as the British Egg Industry Council's Lion code of practice (Anon 1998) put in place in the late 1990s, have had a large impact on reducing the incidence of both human disease and the levels of *Salmonella* on poultry farms (Evans and others 1999, Ward and others 2000, Cogan and Humphrey 2003). Vaccines against *S* Enteritidis and *S* Typhimurium are now widely used in the commercial breeding and layer sectors, and have undoubtedly played a major role in the control of *Salmonella* in poultry flocks (Davies and Breslin 2003a, Anon 2006b).

In 2004/05, all European Union (EU) member states were required to carry out standardised prevalence surveys to establish the baseline

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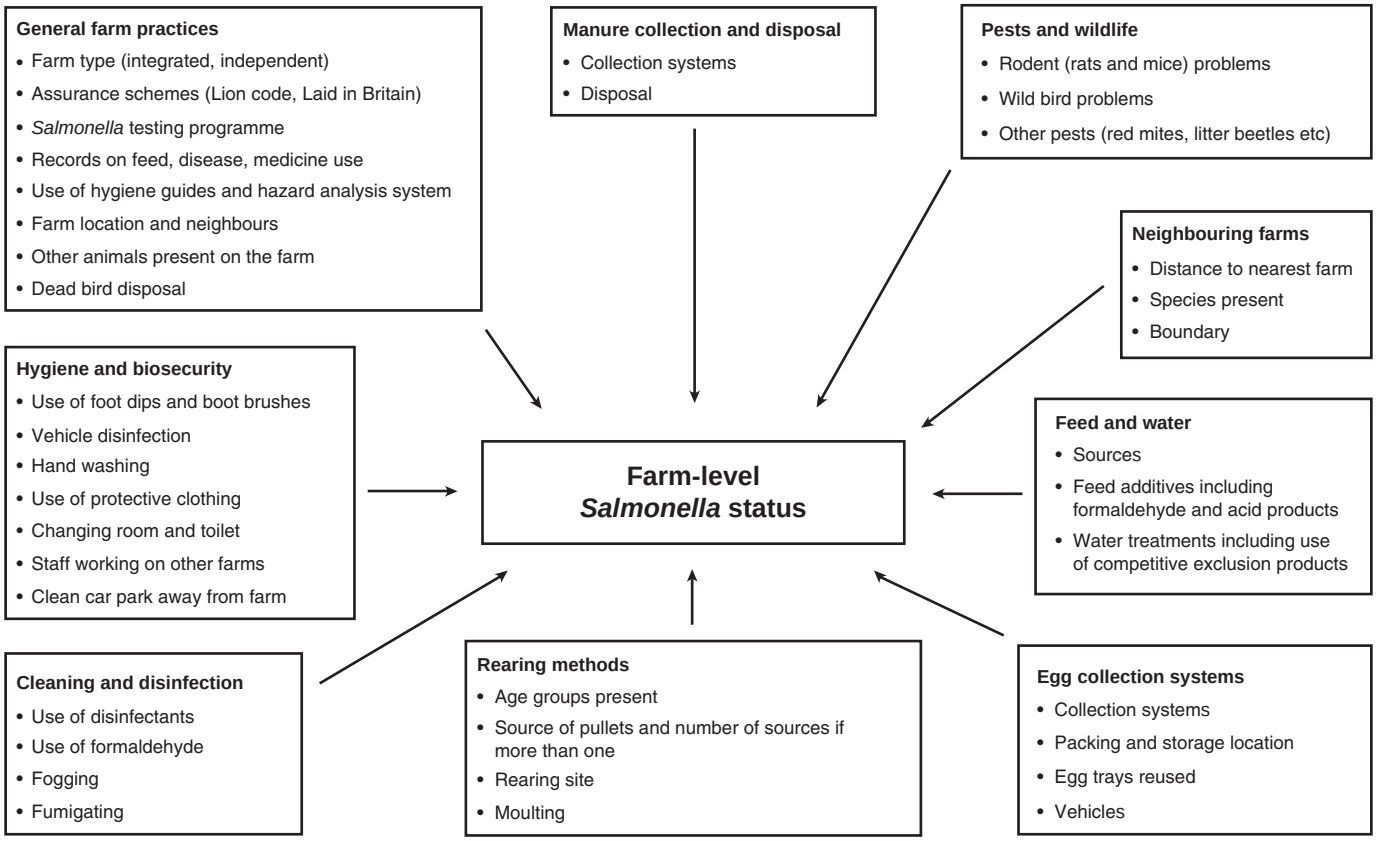

FIG 1: Farm-level and house-level factors on which information was gathered by questionnaires to investigate risk factors for *Salmonella* in 380 laying flocks in Great Britain

prevalence of *Salmonella* in commercial laying flocks. During this survey, the prevalence of *Salmonella* in UK laying farms was estimated at 11.7 per cent (95 per cent confidence interval 9.3 to 14.0 per cent), with *S* Enteritidis present on 5.8 per cent of holdings and *S* Typhimurium present on 1.8 per cent (Snow and others 2007). Although these figures are significantly lower than those for some other EU member states (Anon 2007), they suggest that there are still options for further improvements within the UK laying industry.

The complex epidemiology of *Salmonella* involves human, environmental and animal interactions, and understanding these is key to effective control strategies. The compulsory monitoring and testing of UK breeding flocks (Anon 1993) shows no evidence of significant infection within the layer breeder sector (Anon 2006b). While feed mills and hatcheries remain potential sources of introduction of infection (Veldman and others 1995, Rose and others 1999, Davies and others 2001), the main area of concern remains the long-term persistence of infection on laying farms, largely due to environmental contamination and ineffective cleaning and disinfection between flocks (Davies and Breslin 2003b, Garber and others 2003, Wales and others 2006). These are often coupled with the presence of significant rodent populations, which are involved in the carry-over of *Salmonella* (particularly *S* Enteritidis) between flocks and between houses (Henzler and Opitz 1992, Davies and Wray 1995, Davies and Breslin 2003b). Analytical epidemiological studies have also identified the presence of arthropod pests (Garber and others 2003), the housing system (Mollenhorst and others 2005, Namata and others 2008), flock size (Mollenhorst and others 2005, Namata and others 2008), the presence of birds of different ages on the site (Mollenhorst and others 2005) and age and/or moult (Garber and others 2003, Namata and others 2008) to be important factors in determining the likelihood of *Salmonella* being present in a flock. However, there is a lack of studies examining the risk factors for *Salmonella* in laying hens, and more work is required to build an evidence base to support future control efforts.

During the EU-wide baseline study of *Salmonella* in laying hens in 2004/05, the opportunity arose during the UK survey to collect additional information on farm practices to investigate these potential risk factors in more detail on UK layer farms. This paper describes the results of these analyses.

Materials and methods

The survey design, sampling and laboratory testing methods conformed with the technical specifications set out by the EU (Anon 2004b). A detailed description of the survey design and sampling methods are published elsewhere (Anon 2004b, Snow and others 2007). In summary, a sampling frame of commercial egg-laying holdings in the UK was compiled using information from the British Egg Information Council and the Department of Agriculture and Rural Development, Northern Ireland (DARD). Holdings with fewer than 1000 birds were excluded from the sampling frame. A total of 454 holdings were then selected at random from the list using simple random sampling, stratified by holding size. The number of holdings selected from each stratum was in proportion to the total number of holdings in that stratum. If a holding was later found to be ineligible, it was discarded and another was selected randomly from the same stratum. On each holding, one poultry house was sampled within nine weeks of the end of the laying period (depopulation). For caged houses, five samples of mixed faeces and two dust samples were collected. For barn and free-range houses, five pairs of boot swabs, one dust sample from egg belts and one dust sample from various locations in the house were collected. All the samples were forwarded to the laboratory on the day of collection and kept refrigerated in the laboratory until isolation began within 48 hours of arrival. The *Salmonella* culture method used was a modification of ISO 6579:2002 (Annex D) (ISO 2002).

Basic farm-level information was required by the EU and gathered for all member states using a short questionnaire completed by the Animal Health officer. This included details of the production type and size of the holding, as well as data relating to the house sampled (number of birds, age, crops per year, expected age at slaughter, recent use of vaccines and medicines). In addition, flock owners in the UK were asked to complete an additional voluntary questionnaire, by interview with the Animal Health officer taking the samples. This questionnaire was designed to collect information on additional house- and farm-level

TABLE 1: Results of a univariable analysis of variables associated with the presence of *Salmonella* (any serovar) on 380 farms in Great Britain. Due to the large number of variables significantly associated at P<0.25, only those with P<0.05 are shown

Variable and level	Number of farms sampled	Percentage positive	P (overall chi-squared P value for variable)	Unadjusted OR
Production type				
Cage	120	25.83		1.00
Barn	26	7.69	0.033	0.26
Free-range organic	37	5.41	0.004	0.16
Free-range standard	197	7.11	0.000 (<0.001)	0.24
Holding size (number of birds)				
1000-2999	80	8.75		1.00
3000-4999	47	8.51	0.957	0.97
5000-9999	74	6.76	0.593	0.92
10,000-29,999	113	9.73	0.778	1.12
≥30,000	66	33.33	<0.001 (<0.001)	5.21
Farm affiliation				
Independent farm	318	10.38		
Associated with a company	54	27.78	0.003	3.32
Vaccination status against *Salmonella*				
Not vaccinated	25	32.00		
Sampled flock vaccinated	342	11.40	0.004	0.28
Mice seen				
Rarely/never	263	7.81		
Monthly or more often	64	27.00	<0.001	4.65
Hands washed and/or disinfected between houses				
No	270	14.98		
Yes	108	8.46	0.007	0.45
Houses linked				
No	201	9.45		
Yes	74	27.03	<0.001	3.55
Mains water				
No	51	11.03		
Yes	328	19.53	0.035	0.48
Site all-in/all-out				
No	153	23.53		
Yes	160	7.50	0.010	0.40
House all-in/all-out				
No	44	22.73		
Yes	331	11.48	0.006	0.44
Pullets purchased from an outside source				
No	66	24.24		
Yes	310	10.65	0.001	0.37
Eggs packed on sampled site				
No	148	8.11		
Yes	230	15.65	0.016	2.10
Feed mill				
Company feed mill	61	29.51		
Independent feed mill	117	5.98	<0.001	0.15
Other*	202	11.88	<0.001 (<0.001)	0.32
Neighbouring farms				
Nearest farm ≤1 km	256	14.06		
Nearest farm >1 km	122	9.84	0.105	0.67
Dogs or cats on contiguous farm				
No	170	17.78		
Yes	200	8.50	0.003	0.43
Dogs or cats on sampled farm				
No	92	28.26		
Yes	288	7.99	<0.001	0.22
Clean car park away from house				
No	13	23.08		
Yes	365	12.33	0.108	0.47
Cleaning practices				
No washing or disinfection	20	25.00		1.00
Washing only	15	13.33	0.155	0.46
Disinfection only	31	45.16	0.890	2.47
Washing and disinfection	310	8.39	<0.001 (<0.001)	0.28
Foot cleaning equipment				
None	127	15.75		
Foot dips only	153	15.03	0.546	0.95
Foot dips and boot brushes	100	6.00	0.046 (0.046)	0.34

* National compounder or home-produced
OR Odds ratio

factors that may be related to the risk of *Salmonella,* including information on general farm management, housing, production, feed and pests (Fig 1). The questionnaire was accompanied by guidelines for the interviewer, clarifying the terms used to avoid misinterpretation. All data from the two questionnaires and test results from the sampled holdings in the UK were collated and entered by trained data entry staff into an Access 97 (Microsoft) database at the Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency (VLA) – Weybridge. Data were validated and cleaned and descriptive analysis was carried out to identify errors and outlying observations. Possible data entry errors were checked against the original questionnaires. Variables with 10 per cent or more missing values were excluded.

Statistical methods

All statistical analyses were conducted using Stata Statistical Software Release 9.0 (StataCorp) using the Survey commands for analysing complex survey design data (Levy and Lemeshow 1999, Stata 2005).

Each variable was first examined for an association with holding-level *Salmonella* status based on the Pearson's chi-squared statistic with the Rao and Scott second-order correction, or the Wald test statistic using a weighted logistic regression model (Stata 2005). Any variables significant at $P<0.25$ were assessed for inclusion in the multivariable model.

To provide unbiased estimates of the standard errors, all observations were weighted by the inverse of the selection probability in each holding size stratum (Dargatz and Hill 1996, Levy and Lemeshow 1999). If large proportions of the population are sampled without replacement, variance estimates can be overestimated (Dargatz and Hill 1996, Levy and Lemeshow 1999, Dohoo and others 2004), and as up to 42 per cent of the holdings in some strata were sampled, a finite population correction was also applied to adjust the standard errors accordingly.

Due to the large number of factors under examination, variables were entered into the models manually in a forward stepwise fashion. Variables were included or excluded from the model on the basis of the adjusted Wald test statistic, and only variables with $P<0.05$ were retained (Hosmer and Lemeshow 2000). For variables with multiple levels, one or more needed to be significantly different from the baseline for the variable to be retained. Due to the weighting that was applied to account for the survey design, the likelihood ratio test could not be used to guide variable selection (Levy and Lemeshow 1999). As a final step, variables that were not selected initially were added back individually and retained if significant at $P<0.05$. The fit of the final model was assessed by removing the weightings and using the Hosmer-Lemeshow goodness of fit test (Hosmer and Lemeshow 2000).

Possible confounders such as the holding size, production type, belonging to an integrated company, membership of quality assurance schemes or the age of birds on the holding were investigated and included in the model if they were either associated with the risk factor, associated with flock-level *Salmonella*, biologically meaningful as confounders, or if they caused a biologically important change in the odds ratio (OR) (approximately 10 per cent) of

TABLE 2: Results of a univariable analysis of variables associated with the presence of *Salmonella* Enteritidis on 358 farms in Great Britain. Due to the large number of variables significantly associated at P<0.25, only those with P<0.05 are shown

Variable and level	Number of farms sampled	Percentage positive	P (overall chi-squared P value for variable)	Unadjusted OR
Production type				
Cage	109	18.36		
Non-cage (including barn)	249	2.81	<0.001	7.77
Holding size (number of birds)				
1000-2999	76	3.95		
3000-9999	114	1.74	0.294	0.43
10,000-29,999	107	4.67	0.776	1.19
≥30,000	61	27.87	<0.001 (<0.001)	9.40
Vaccination status against *Salmonella*				
Not vaccinated	22	2.81		
Vaccinated	324	18.35	0.005	0.24
Farm affiliation				
Independent farm	303	5.94		
Associated with a company	47	17.02	0.002	3.25
Foot dips on entry to the sampled house				
No	166	10.25		
Yes	192	5.21	0.016	0.48
Hands washed and/or disinfected between houses				
No	219	9.49		
Yes	137	2.91	0.010	0.34
Houses linked				
No	191	4.71		
Yes	71	23.94	<0.001	6.34
Pullets purchased from an outside source (different owner from the sampled farm)				
No	60	16.67		
Yes	294	5.78	0.001	0.31
Pullets reared on another site				
No	302	11.94		
Yes	49	1.33	<0.001	4.58
Eggs packed on sampled site				
No	138	1.45		
Yes	218	11.01	<0.001	8.41
Egg transported and packed in separate packing plant				
No	185	9.73		
Yes	169	4.73	0.017	0.46
Water source				
Mains	300	5.03		
Bore	46	17.98	0.001	4.05
Cats or dogs present on contiguous farm				
No	167	11.38		
Yes	191	4.19	0.005	0.34
Rodenticides used				
No	316	6.01		
Yes	37	21.62	<0.001	4.31
Flies present				
No	147	3.40		
Yes	201	9.95	0.006	3.14
Feed mill used				
Company feed mill	53	18.18		
Independent feed mill	114	3.51	0.001	0.16
Other	191	6.81	0.003 (0.001)	0.31
Mice seen				
Rarely/never	272	3.31		
Monthly or more often	82	21.95	<0.001	8.21
Rats seen				
Rarely/never	286	6.23		
Monthly or more often	65	13.85	0.037	2.42
Dogs or cats on farm				
No	83	20.48		
Yes	275	3.64	<0.001	0.15
Site all-in/all-out				
No	201	11.94		
Yes	150	1.33	<0.001	0.10

OR Odds ratio

the risk factor when included in the model. All the variables in the final models were assessed for biologically plausible interactions; however, due to a small number of positive samples in some strata, this was not possible in all cases.

Two separate models were developed. For the first model, the outcome was the detection of *Salmonella* (any serovar). In the second model, the outcome was detection of *S* Enteritidis versus farms testing negative for any *Salmonella*. It was felt that these two outcomes were of most interest in terms of realistic farm-level interventions. Ideally, interventions targeting *S* Enteritidis would also have an impact on other salmonellae present on the farm, and through the exclusion of non-*S* Enteritidis-infected farms in the second model it was hoped that the model would identify factors that, while most strongly related to *S* Enteritidis, might also impact on other serovars, if present.

The population attributable fraction (PAF) is a measure of the proportion of disease in the whole population that is attributable to exposure to a specific risk factor, and so would theoretically be avoided if that risk factor was completely eliminated (Dohoo and others 2004). This is a function of the strength of the association and the prevalence of the exposure, and can be useful in determining which measures may have the greatest effect in reducing prevalence in the population if they are removed. Because the calculation of attributable fractions relies on measures of risk (risk ratios) and the logistic regression employed here yields ORs, risk ratios were estimated using the formula described by Zhang and Yu (1998) and used as the measure of risk for this step of the analyses. Adjusted PAFs were calculated, where appropriate, for statistically significant variables to examine what effect removal or changes in these variables might have on *Salmonella* in the population, using the methods outlined by Bruzzi and others (1985). Confidence intervals based on the estimated upper and lower bounds of the PAFs were calculated, based on the upper 90 per cent and lower 90 per cent confidence limit of the ORs for each stratum, as described by Wells and others (1996) and Kabagambe and others (2000). Since it is unlikely that for all variables each stratum would be at the highest level simultaneously, the 90 per cent confidence limit is used instead of the 95 per cent confidence limit (Wells and others 1996).

Results

Response to voluntary questionnaire

A total of 380 (83.7 per cent) of the 454 farmers enrolled in the EU survey completed the additional questionnaire on risk factors. A comparison of these 380 farms with the 454 that completed the compulsory data collection form showed no significant differences in terms of production type, *Salmonella* status, vaccination status, or number of other flocks on site (data not shown). No farmers from Northern Ireland completed the additional questionnaire, but from those in Great Britain there was no significant difference in response by devolved region (England, Scotland and Wales). Holdings with between 5000 and 9999 birds were less likely to fill out the questionnaire, with a 73 per cent response, rate while the largest holdings (>30,000 birds) were most likely to complete it (93 per cent response rate). This was a small but significant difference (P=0.012), and as under- or oversampling in particular strata were taken into account using the weighted approach described above, it was not considered to introduce unacceptable bias into the analyses.

TABLE 3: Factors associated with farm-level *Salmonella* status (all serovars) and the population attributable fractions (PAFs) for variables significant in the final model

Variable	Number of holdings (n=354)	OR	95% CI	P_{wald} (overall P value for variable)*	PAF†	Confidence limits
Production type						
Cage	114	1.00			0.56	−0.24-0.79
Barn	24	0.31	0.07-3.35	0.120		
Free-range organic	34	0.43	0.08-2.24	0.318		
Free-range standard	188	0.75	0.27-2.11	0.590 (0.378)		
Holding size (number of birds)						
1000-2999	75	1.00			0.45	−0.35-0.70
3000-4999	45	1.03	0.25-4.21	0.963		
5000-9999	69	1.29	0.30-5.60	0.737		
10,000-29,999	108	2.15	0.62-7.46	0.228		
≥30,000	63	4.79	1.22-18.78	0.025 (0.050)		
Sampled flock vaccinated against *Salmonella*						
No	24	1.00				
Yes	336	0.28	0.10-0.76	0.013	0.11‡	0.06-0.13
Feed mill						
Company feed mill	57	1.00				
Independent feed mill	108	0.19	0.06-0.64	0.007	0.53	0.03-0.73
Other	195	0.40	0.16-0.99	0.049 (0.018)		
Nearest farm						
<1 km	244	1.00			0.41	0.13-0.55
1-2 km	116	0.45	0.23-0.88	0.021		
Dogs or cats on contiguous farm						
No	171	1.00			0.37	0.12-0.50
Yes	189	0.44	0.21-0.92	0.03		
Dogs or cats on sampled farm						
No	88	1.00			0.35	0.22-0.41
Yes	272	0.26	0.13-0.63	0.002		
Clean car park away from house						
No	13	1.00			0.06‡	0.04-0.07
Yes	347	0.14	0.05-0.44	0.001		
Cleaning practices						
No washing or disinfection	20	1.00				
Washing only	14	0.14	0.03-0.62	0.01		
Disinfection only	29	0.36	0.10-1.33	0.125		
Washing and disinfection	297	0.19	0.06-0.56	0.003 (0.018)	0.21‡	−0.19-0.33
Foot cleaning equipment						
None	118	1.00				
Foot dips only	146	0.40	0.15-1.02	0.055		
Foot dips and boot brushes	96	0.27	0.08-0.96	0.042 (0.081)	0.42‡	−0.21-0.66

* P value for test of linear hypothesis for variables with multiple levels
† PAF is calculated as the proportional reduction in *Salmonella* prevalence that would occur if all farms had the lowest risk level of the factor
‡ Combined PAF if all farms carried out measures to reduce these factors to the lowest risk level

Factors associated with farm-level *Salmonella* status

Univariate analysis

A total of 65 (*Salmonella* species model) and 63 (*S* Enteritidis model) variables were significant at P<0.25 and were considered for inclusion in the multivariable models; not all can be listed here, and only the strongest associations are described in Tables 1 and 2. Table 1 shows the variables significantly associated with *Salmonella* species in the univariate analysis where P<0.05 or where the variable was included in the final model. The same information is shown in Table 2 for *S* Enteritidis.

Multivariable results

Tables 3 and 4 show the final multivariable model with adjusted ORs and 95 per cent confidence intervals for factors associated with Salmonella species (Table 3) and S Enteritidis (Table 4) at farm level. Also listed are the adjusted PAFs, and PAF confidence limits for each variable. Risk ratios estimated using the method described by Zhang and Yu (1998) have not been shown and differences between adjusted ORs and estimated risk ratios were minimal, although they did increase slightly as the ORs became large.

Factors associated with farm-level *Salmonella* status (all serovars)

Holding size and production type were found to be associated with both *Salmonella* and a number of other variables at the univariable level so were retained as confounders in the final model. Holding size was not significantly associated with *Salmonella* except in the largest holding size category of 30,000 or more birds, in which there an increased risk of the holding testing positive for *Salmonella* (OR 4.79) compared with holdings of between 1000 and 2999 birds (Table 3).

Farms that used a company feed mill (owned by the poultry company that owned the farms or to which the site was contracted) were more likely to be positive for *Salmonella*, compared with farms that used an independent feed mill (not part of a national organisation) (OR 0.19) or another source of feed (OR 0.40) (Table 3). This second group was composed mainly of farms using a national compounder (part of a major national group of feed mills) or home-produced feed. Despite a univariate association between farm affiliation and *Salmonella,* this variable was not retained in the final model and showed no evidence of being a confounder.

A large proportion (93 per cent) of the sampled farms vaccinated against *Salmonella*, and vaccination of the sampled flock significantly reduced the likelihood of finding *Salmonella* in the house (OR 0.28). Holdings where the sampled house was washed (OR 0.14) or washed and disinfected (OR 0.19) or where foot dips and brushes were used (OR 0.27) were significantly less likely to test positive for *Salmonella.* Having a clean car parking area away from the houses (OR 0.14), the presence of cats and dogs on the farm (OR 0.26) or on contiguous farms (OR 0.44), and the nearest farm being over 1 km away (OR 0.45) were also protective for *Salmonella*; no interaction was detected between the distance to the nearest farm and the effect of having dogs or cats on the contiguous farm.

Factors associated with farm-level *S* Enteritidis status

Of the 49 *Salmonella*-positive farms, 27 (55 per cent) tested positive for *S* Enteritidis in one or more samples. Thus, 22 farms that were positive for *Salmonella* but did not test positive for *S* Enteritidis on any samples were excluded from the analysis, leaving 358 farms for inclusion in the second model. Table 4 shows the final multivariable model for factors associated with *S* Enteritidis at farm level, and the adjusted PAFs for each variable.

Due to the smaller number of farms included and fewer positive farms, it was necessary to collapse a number of the variable levels. There was a significantly lower risk of *S* Enteritidis in non-caged birds (barn and free-range) than in caged birds (OR 0.14) (Table 4). In addition, holdings with 30,000 or more birds were over 14 times as likely to test positive for *S* Enteritidis as those with 1000 to 2999 birds.

Vaccination of the sampled house was protective against *S* Enteritidis (OR 0.08). This effect was stronger when considering *S* Enteritidis alone than in the model looking at all *Salmonella* serovars, which may be expected, as the majority of the vaccines used specifically targeted *S* Enteritidis.

Using a company feed mill was associated with an increased risk of *S* Enteritidis (Table 4); farms that used using neither a company nor an independent feed mill (that is, those producing feed at home or using a national compounder) were at lowest risk (OR 0.11) even after controlling for whether the farm was either owned or contracted to rear birds by a poultry company. Furthermore, in this model, affiliation with a company seemed to be related to a reduced probability of

TABLE 4: Factors associated with holding-level *Salmonella* Enteritidis status and the population attributable fractions (PAFs) for variables significant in the final model

Variable and level	Number of holdings (n=327)	OR	95% CI	P_{Wald} (overall P for variable)*	PAF†	Confidence limits
Production type						
Cage	100	1.00				
Non-cage (including barn)	227	0.14	0.04-0.49	0.002	0.59	−0.02-1.20
Holding size (number of birds)						
1000-2999	70	1.00			0.61	0.29-0.93
3000-9999	103	0.72	0.13-4.15	0.715		
10,000-29,999	99	1.86	0.42-8.30	0.416		
≥30,000	55	14.88	3.16-70.08	0.001 (0.017)*		
Farm affiliation						
Independent farm	283	1.00				
Associated with a company	44	0.14	0.03-0.74	0.020	0.49	0.16-0.30
Sampled flock vaccinated						
No	21	1.00			0.17‡	0.01-0.33
Yes	306	0.08	0.02-0.38	0.001		
Feed mill						
Company feed mill	48	1.00	1.00			
Independent feed mill	103	0.49	0.11-2.14	0.343		
Other	176	0.11	0.03-0.47	0.003 (0.011)*	0.09	−1.69-1.88
Dogs or cats on sampled farm						
No	77	1.00			0.54	−0.04-1.12
Yes	250	0.14	0.04-0.50	0.002		
Mice seen						
Less frequently than monthly	253	1.00			0.50‡	−0.29-1.29
Monthly or more often	74	5.78	1.65-20.18	0.006		
Rats seen						
Less frequently than monthly	266	1.00			0.31‡	−0.18-0.81
Monthly or more often	61	8.17	2.75-24.34	0.000		
Site						
Not all-in/all-out	186	1.00				
All-in/all-out	141	0.06	0.01-0.24	0.000	0.85	0.17-1.54

* P value for test of linear hypothesis for variables with multiple levels
† PAF is calculated as the proportional reduction in Salmonella prevalence that would occur if all farms had the lowest risk level of the factor
‡ Combined PAF if all farms carried out measures to reduce these factors to the lowest risk level = 0.97
CI Confidence interval, OR Odds ratio

testing positive for *S* Enteritidis (OR 0.14) after controlling for other factors in the model, and showed evidence of being a confounder in the association between feed mill and *Salmonella*.

The presence of rodents on the farm increased the risk of *S* Enteritidis if they were seen monthly or more often, and this was true for both rats (OR 8.17) and mice (OR 5.78). Running the whole site as all-in/all-out (OR 0.06) and the presence of dogs or cats on the farm (OR 0.14) reduced the likelihood of having *S* Enteritidis (Table 4). No association was found between the presence of *S* Enteritidis on the farm and the cleaning and disinfection routine used, once other factors had been controlled for in this model.

PAFs

Tables 3 and 4 show the adjusted PAFs and their confidence limits associated with each significant risk factor for *Salmonella* species and for *S* Enteritidis.

The PAFs for factors associated with *Salmonella* species (Table 3) range from 0.06 to 0.56, and estimate the proportion reduction of disease in the population that might be achieved if the risk factor were removed. Thus, the proportion of current *Salmonella* that would be removed from the population if all flocks were vaccinated is approximately 11 per cent (Table 3). This is low because 98 per cent of the flocks were vaccinated anyway, so the impact of changing this risk factor would be low. The PAF of *S* Enteritidis due to non-vaccination is higher, at 17 per cent, suggesting that although more extensive vaccination of flocks may have a small impact on *Salmonella* overall, it could play a larger role in improved *S* Enteritidis control.

To estimate what the overall effect on *Salmonella* would be if all farms washed and disinfected the poultry houses after depopulation (as opposed to washing alone, disinfection alone, or nothing), vaccinated flocks, used boot brushes together with foot dips, and had a clean parking area away from the houses, the adjusted combined PAF (Bruzzi and others 1985) for these factors was calculated, assuming all other factors remained the same. Such an intervention would lead to an approximate reduction of *Salmonella* in the population of 60 per cent.

The PAFs for the presence of mice and rats in the *S* Enteritidis model (Table 4) were 50 per cent and 31 per cent, respectively, suggesting that effective rodent control may significantly reduce the amount of *S* Enteritidis in the population. To examine the joint effect of multiple interventions on *S* Enteritidis, an adjusted PAF was calculated for the reduction of disease in the population if effective rodent control (mice and rats) was carried out, the site was run as all-in/all-out if not already done so, and all flocks were vaccinated, but all other factors remained the same. This intervention would theoretically lead to a 97 per cent reduction of the current infection levels in the population.

The confidence limits for many of the PAFs were broad. Negative PAFs were interpreted as the risk factor being protective, that is, suggesting that higher infection rates would be caused by the removal of the risk (Wells and others 1996).

Discussion

This study used data gathered from a sample of commercial laying farms in Great Britain to identify a number of factors that are likely to be related to farm-level *Salmonella* status.

The size of the holding was consistently associated with an increased risk of infection in both the *Salmonella* species and *S* Enteritidis models; this supports the results of other studies in which the size of the flock has been shown to be important (Mollenhorst and others 2005; Namata and others 2008). No effect of the type of production was noted when *Salmonella* was the outcome; however, caged systems were a significant risk for *S* Enteritidis infection. This confirms the findings of the EU-wide survey with regard to *S* Enteritidis (Anon 2007) and also recent work in Belgium (Namata and others 2008) and the UK (Carrique-Mas and others 2009). This is probably a result of a close association between rodents and caged systems.

Both final multivariable models found a significant protective effect of vaccination for both *Salmonella* overall and *S* Enteritidis. Vaccination has been advocated as a method of *Salmonella* control on farms for many years, and its practical efficacy has been supported by other studies (Feberwee and others 2001, Davies and Breslin 2003a). Despite the strength of these associations, a large proportion of flocks in the present survey (over 98 per cent) already vaccinated against *Salmonella*, and vaccinating the remaining flocks was shown to have a small effect on lowering the overall *Salmonella* prevalence (all serovars) from current levels in the population. However, increasing the proportion vaccinated was shown to have a larger impact on *S* Enteritidis, reducing the amount of infection in the population by approximately 17 per cent.

Although this study consistently found an association between the use of company feed mills and an increased likelihood of testing positive for *Salmonella*, the most common serovars identified in this study (on 69 per cent of positive holdings) were *S* Enteritidis and *S* Typhimurium, neither of which was among the top six serovars isolated from feedstuffs in 2004 or 2005 (Anon 2006b). This suggests that feed is not currently an important route of transmission for *S* Enteritidis or *S* Typhimurium, although both have been isolated from sites on broiler company feed mills. Thus, although rare, contamination with these serovars may occur (Davies and Wray 1997, Davies and others 2001). Of the other serovars associated with feed in 2004 or 2005 (Anon 2006b), only *S* Yoruba, *S* Mbandaka and *S* Livingstone

were found on the holdings in this study, and on only six holdings in total, so were unlikely to be responsible for the association between company feed mills and *Salmonella*. Company feed mills in the layer industry are often situated on large laying farms where cross-contamination can occur (R. H. Davies, personal communication). It is recognised that some companies do have persistent problems with *Salmonella* contamination, which is intensified by the relatively closed networks and carry-over of infection on farms or dissemination on fomites from the hatchery or personnel (Davies and others 2001).

In analytical cross-sectional studies such as that described here, it is difficult to differentiate between risk factors that reflect an increased risk of introduction of infection from those that increase the ability of the organism to persist on a site. Both will be detected in this type of study. In order to separate these factors, detailed longitudinal or cohort studies would be required, and this should be borne in mind when interpreting risk factors from this work.

Good cleaning and disinfection practice has previously been shown to be effective in reducing *Salmonella* overall (Davies and Breslin 2003b, Garber and others 2003). Interestingly, disinfection alone without additional washing was not shown to have a significant effect on *Salmonella* in the present study, possibly due to the failure of disinfection to penetrate organic matter left in the houses (Davies and Breslin 2003b, Wales and others 2006). This is also reflected in the finding that boot brushes used in conjunction with foot dips were most significantly associated with a reduced risk of *Salmonella*. Disinfectants in foot dips may not effectively kill bacteria; however, using a boot brush to remove organic matter from the boots may be more effective (Amass and others 2000). Cleaning and disinfection did not appear to have a significant impact on *S* Enteritidis, despite showing an effect for all *Salmonella* serovars. The persistence of *S* Enteritidis has been linked to the presence of significant rodent populations (Henzler and Opitz 1992, Davies and Wray 1995, Garber and others 2003); even if effective cleaning and disinfection are carried out, reintroduction into the house is likely if infected rodents are present (Rose and others 2000, Wales and others 2006). Reporter bias is a particular issue in studies that rely on questionnaires to gather information, and it was unclear in the present study how accurately practices such as cleaning and disinfection, adherence to biosecurity policies or the frequency of rodent sightings were recalled and recorded.

Although various aspects of overall cleanliness and good hygiene have been examined in this study, these are all interlinked and it is stressed that one of the key factors in maintaining low levels of *Salmonella* on farms is a comprehensive management programme tackling cleaning, hygiene, pest control and biosecurity, which must be maintained. Follow-up studies carried out on some of these farms showed that despite cleaning and disinfection, *Salmonella* (including *S Enteritidis*) was still present in most of the cage houses when the next flock was introduced (Carrique-Mas and others 2008). The persistent contamination of some poultry holdings poses significant problems when attempting to control *Salmonella*.

There were a number of unexpected findings in the present analysis. Both final multivariable models suggested that the presence of cats and dogs reduced the risk of *Salmonella* being present. This could be because cats and dogs may play a role in deterring rodent populations or keeping wild birds away from poultry houses and the surrounding area. However, the authors do not consider that keeping cats or dogs on farms should be promoted as a means of controlling *Salmonella*.

PAFs allow an estimation of the proportion of infection in the population that may be prevented by effectively reducing risk factors to the lowest level, assuming that there is a causal link between the risk factors and the infection. The PAFs for factors such as holding size, production type, distance to nearest farm and feed source (Table 3) are less informative, as little can be done about the risks associated with these variables until information is available about how they influence *Salmonella* on farms. Furthermore, many of the confidence limits for the PAFs were very broad, but although a number of methods for estimating confidence limits for PAFs have been proposed (Wells and others 1996, Wagner and others 2001), there are no methods for estimating the exact variance of these parameters in the context of complex survey analyses (Wells and others 1996).

PAFs assume a causal link between risk factors and disease. While causality is assumed between these factors and *Salmonella* in order to interpret the PAFs, it is impossible to verify a causal role through cross-sectional studies such as the present study, and caution is needed when interpreting the PAFs. However, such measures can be informative for policy decisions or when assessing the effect of combinations of interventions. Combinations such as improved cleaning and disinfection, vaccination, the use of foot dips and brushes, and improved rodent control could have significant effects on reducing *Salmonella* levels on farms in Great Britain, and the findings of the present study support previous work emphasising the importance of maintaining good farm biosecurity, hygiene practices and pest control in reducing levels of *Salmonella* on layer farms (Davies and Breslin 2003b, Garber and others 2003, Wales and others 2006).

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